

PRELIMINARY OBSERVATIONS ON BACTERIOLOGICAL QUALITY
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PRELIMINARY OBSERVATIONS ON BACTERIOLOGICAL QUALITY

OF FRESH N. C. BLUE CRAB MEAT**

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Relatively little information is available on the bacteriology of fresh crab meat from the Atlantic blue crab (*Callinectes sapidus*) as it is delivered to the consumer, and none is available on crab meat processed in North Carolina. Furthermore, information on procedures and methods for the evaluation of the bacteriological quality is exceptionally limited. In order to propose standards for quality control or regulation, data had to be obtained on the present bacteriological condition of the North Carolina product. In addition, projected studies on pasteurization of crab meat necessitated the selection of adequate procedures and the evaluation of the raw material. Therefore, a project was initiated to select suitable methods for the bacteriological analyses of crab meat products and to determine the levels of bacterial populations in the fresh product immediately after processing and during refrigerated storage.

Table 1. Influence of sampling on apparent bacteriological quality of fresh crab meat. Crabs processed on same day, cans selected at random, total colony count obtained with Plate Count Agar at 25°C.

Position of Crab Meat in Container	Counts x 10 ⁴ /g		
	Can 1	Can 2	Can 3
Top	3.9	2.9	17
Center	3.6	2.3	13
Bottom	3.7	2.7	12

*Presented by F. B. Thomas at 10th Annual Atlantic Fisheries Technological Conference, Charlottetown, Prince Edward Island, Canada, October 3 - 6, 1965.

**Distribution of this report does not constitute publication. The data contained herein are preliminary and are subject to correction and/or revision.

Table 1 presents data indicating the influence of sampling on the apparent bacteriological quality of fresh crab meat. Three one pound containers were selected at random at one processing plant, three areas within the container were sampled. These counts were obtained by blending 50 grams of fresh crab meat with 3% NaCl diluent and making serial dilutions with the same diluent. Previous studies in our laboratory had indicated that blending of the sample resulted in higher counts than those obtained when the crab meat was dispersed in the diluent by shaking with glass beads. Also, previous studies suggested that when NaCl solution, phosphate buffer, and distilled water were compared, diluent had little or no effect on subsequent counts. Bacto-Plate Count Agar (Difco) was used as the plating medium and the plates were incubated at 25°C for 7 days after which time additional incubation did not increase counts. Incubation at 32°C consistently resulted in counts that were lower than those obtained at 25°C. These data presented in this table indicate that total colony counts obtained from selected areas within a given container were essentially equal; however, considerable variation was observed among counts obtained from each container.

Table 2. Apparent bacteriological quality of several grades of crab meat. Crab processed on same day, cans selected at random, total colony count obtained with Plate Count Agar at 25°C.

<u>Sampling</u>	GRADE		
	Count x 10 ³ /g		
	<u>Backfin (lump)</u>	<u>Special (Flake)</u>	<u>Claw</u>
1	7.3	24	4.3
2	5.0	27	10
3	4.0	15	7.5
4	5.0	14	25
5	3.5	19	--
6.	3.6	6.0	--

Table 2 shows the variation in total colony counts among types or grades of crab meat. Each sampling was made on products from a selected plant on a specific day in an attempt to obtain samples of all three types of crab meat from one batch of crabs processed under similar conditions. Backfin or lump grade consistently had the lowest total count and special grade (white, regular, or flake) generally had the highest total count. Backfin meat can be removed from the crab in one piece, but special meat is removed in many pieces, and we feel that the additional handling contributes to the higher bacterial counts. Variation in claw meat count can be attributed to the variation in manual dexterity among pickers.

Table 3. Influence of source and season on total colony count on fresh N. C. Blue Crab Meat (special grade) obtained with Plate Count Agar at 25°C.

Plant	Month	Total colony count x 10 ⁴ /g fresh crab meat
A	Jan.	0.92
	May	1.1
	June	0.57
	June	3.9, 2.6, 14.
	Aug.	3.8
B	Feb.	2.4
	Mar.	1.4
	Apr.	2.0
C	Mar.	1.6
	Apr.	1.1
D	May	4.7
	June	13

Table 3 indicates the influence of source and season on the total bacterial count observed in samples of fresh crab meat obtained from several plants during several months of 1965. No count from a given plant was unusually high or low; however, the counts from samples of Plant A generally were lower than the average. Subjective evaluation of the sanitation program at this plant suggested little room for improvement. The counts from samples of Plant D generally were higher and in turn, evaluation of sanitation at Plant D indicated considerable room for improvement.

Aside from cleanliness, in-plant handling practices were noted to vary widely. Often when large amounts of cooked and cooled crabs were placed on the picking tables some individual crabs could be left at elevated temperatures for extended periods of time. This condition would lead to higher bacterial counts than normal. With a rapid turn-over of cooked crabs bacterial counts could be lowered appreciably.

Figure 1. (Plate count per gram versus Storage time). As expected, increases in total counts were observed during refrigerated storage of fresh samples. When total colony counts approached 10⁹ per gram, after 13 days storage, the crab meat was completely unacceptable for consumption. These data indicate that the crab meat is sufficiently contaminated with psychrophilic organisms to limit refrigerated storage to not more than two weeks.

When plating media were evaluated during the study, Plate Count Agar was found to be inadequate for the enumeration of psychrophilic organisms which had proliferated on the crab meat, and Trypticase Soy Agar (BBL) was found to be the most productive of several which were investigated. Other media that were studied included nutrient agar fortified with glucose and minerals, glucose minimal salts medium, and crab infusion agar formulated in our laboratory. (Table 4.)

Figure 2. (Plate count per gram on Two Media versus Storage time.) This figure presents data on samples stored at 3°C up to 10 days. The total population was determined by colony count with either Plate Count Agar or Trypticase Soy Agar. Each point represents an average of counts from the number of samples indicated at the top of the figure. Initially, counts at 25°C on either medium were essentially the same. However, as the period of storage progressed and the psychrophilic organisms increased in number, the Trypticase Soy Agar enumerated two to four times as many organisms as were observed with the other medium. Although Plate Count Agar initially appeared to be adequate, evaluations of stored samples of crab meat indicated that Trypticase Soy Agar was the medium of choice.

Figure 3. (Effect of Incubation temperature with Two Media.) Counts of organisms that had grown at 3°C and that were enumerated at 25°C with Trypticase Soy Agar exceeded counts at 25°C with Plate Count Agar. As can be seen in this figure, differences in counts obtained at 6°C with the two media were even greater than the differences observed with 25°C incubation. Again, on fresh samples both media were equally productive at 6°C or 25°C. The reasons for the enhanced colony formation on Trypticase Soy Agar are not readily evident, but additional study may elucidate the specific nature of the psychrophiles which allows them to grow more readily on the medium without glucose.

Regardless of the reason for the differential in count on the two media, this differential may prove to be an excellent tool for the evaluation of the freshness of blue crab meat and other seafoods since this difference in counts appears after storage and increases with storage time.

Admittedly methods employing counts at 25°C requiring incubations for at least three days do not lend themselves to routine production evaluations. Nevertheless, these methods not only could be used in evaluation samples on a long term basis, but also should prove valuable as a research tool in studies on pasteurization of crab meat.

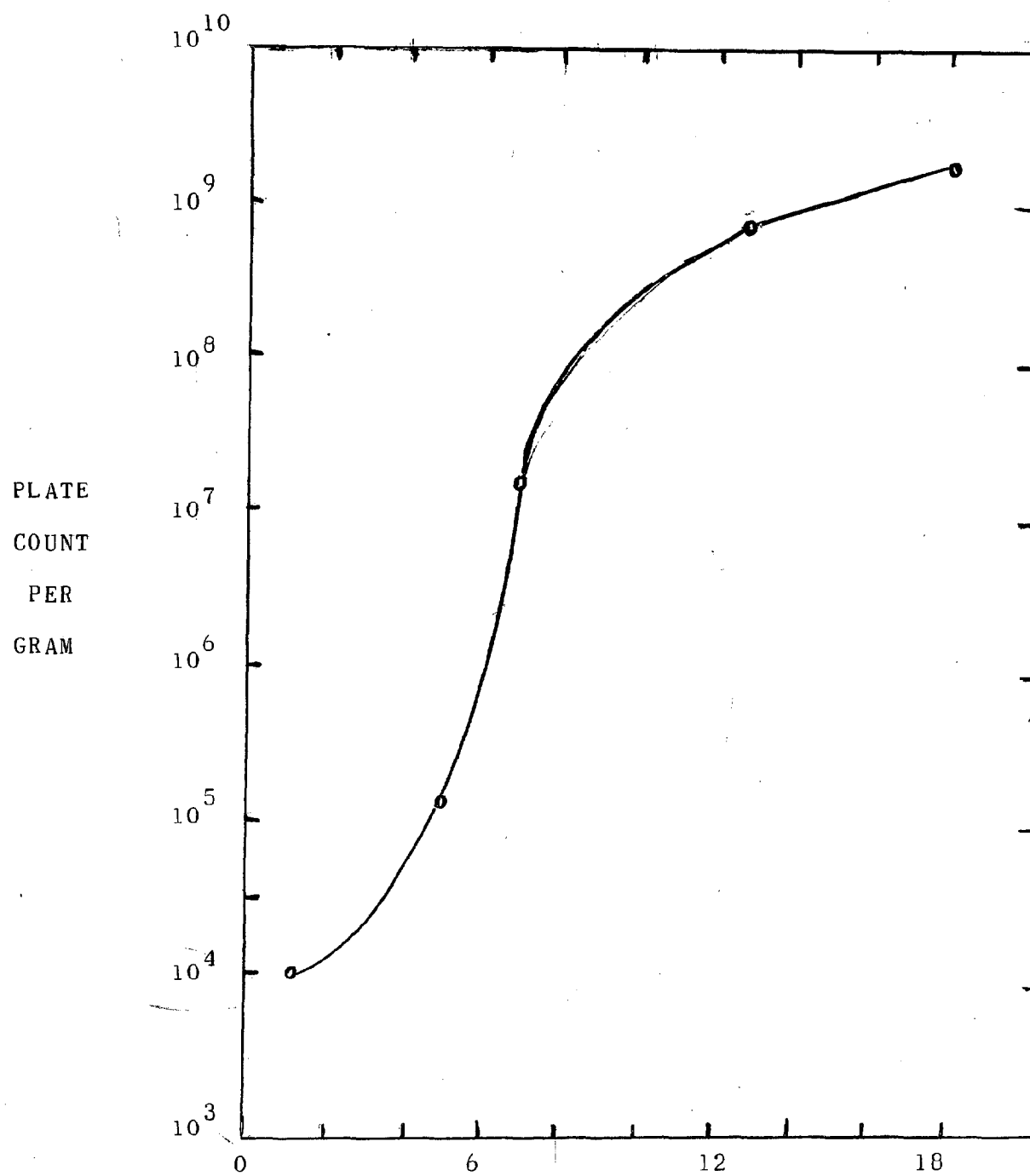


FIGURE 1

Plate Count per gram versus storage time

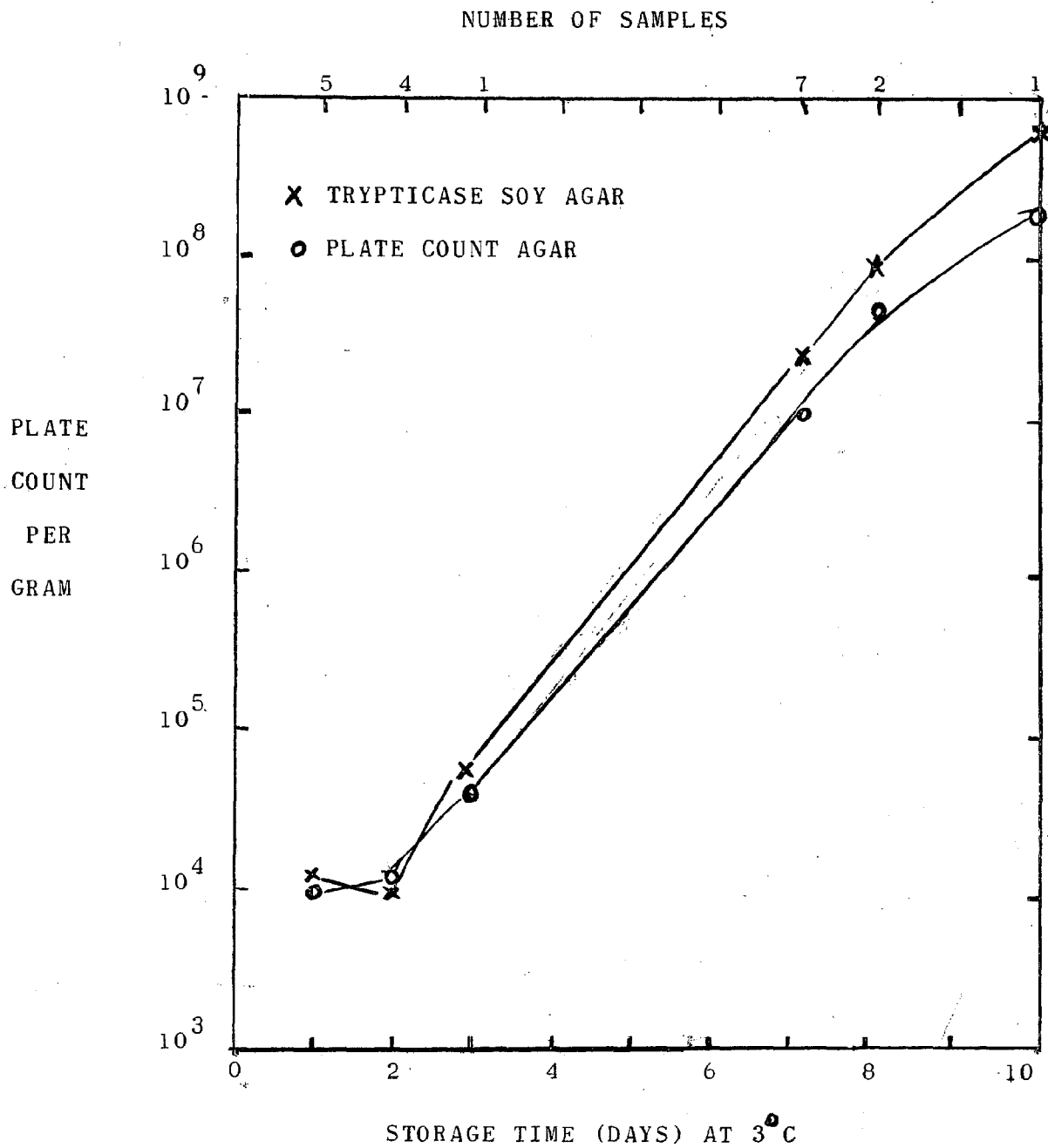


FIGURE 2

Plate count per gram on Two Media versus storage time.

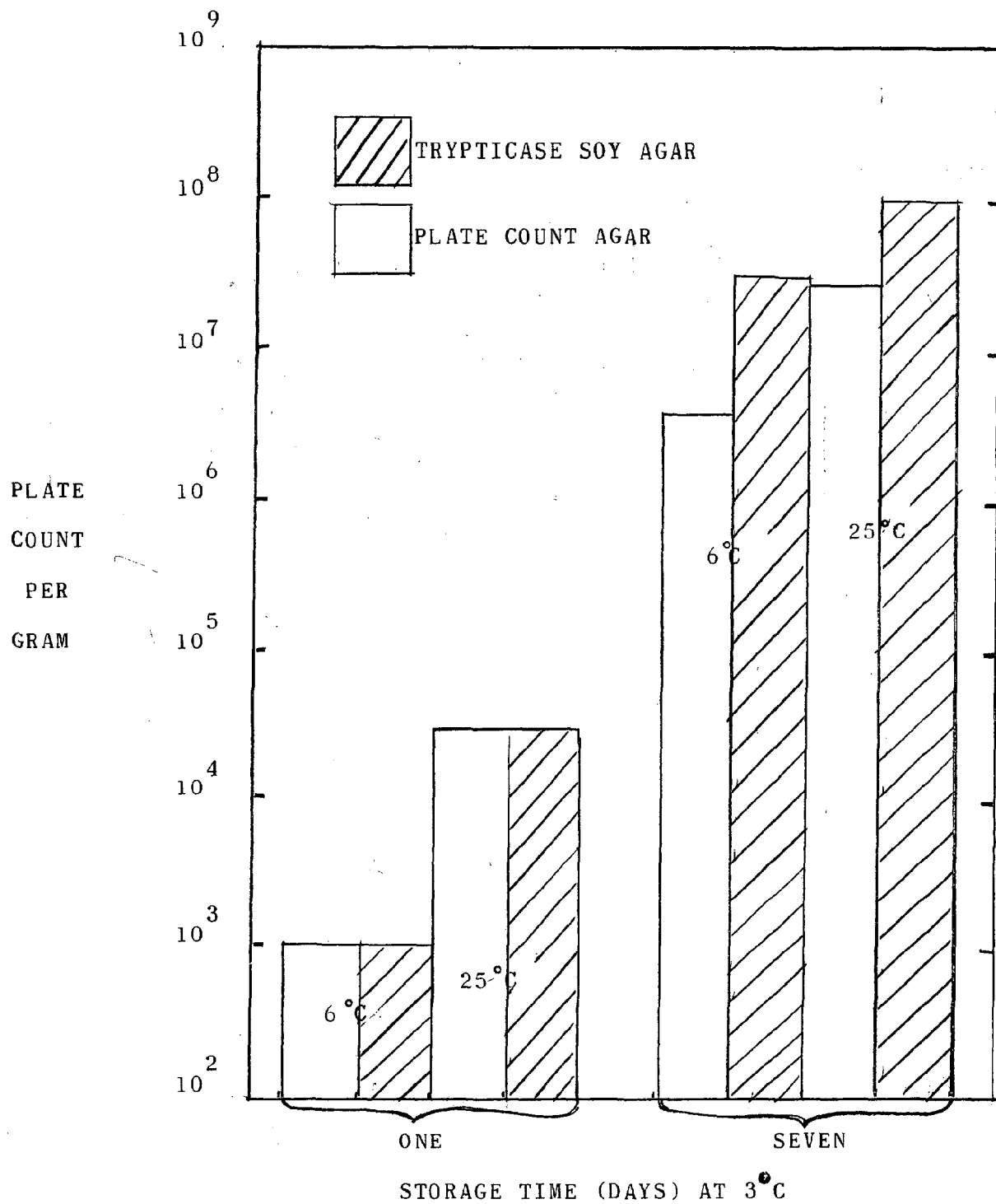


FIGURE 3

Effect of Incubation temperature with Two Media

Table 4.

MEDIA USED FOR CRAB MEAT STUDIES

Plate Count Agar

g./l.

- | | | |
|-----|--------------------------|------|
| (1) | Bacto-Beef Extract ----- | 3g. |
| (2) | Bacto-Peptone ----- | 5g. |
| (3) | Bacto Agar ----- | 15g. |

Trypticase Soy Agar

g./l.

- | | | |
|-----|--|------|
| (1) | Trypticase (B-B-L Pancreatic-Digest of Casein) ----- | 15g. |
| (2) | Phytone (B-B-L Soy Peptone) ----- | 5g. |
| (3) | Sodium Chloride ----- | 5g. |
| (4) | Agar ----- | 15g. |

Fortified Nutrient Agar

g./l.

- | | | |
|-----|-------------------------|---------|
| (1) | Nutrient Agar ----- | 23g. |
| (2) | Agar ----- | 5g. |
| (3) | NaCl ----- | 8g. |
| (4) | Glucose ----- | 0.1g. |
| (5) | CaCl ₂ ----- | 0.08g. |
| (6) | Gbl minerals ----- | 100 ml. |
| (7) | H ₂ O ----- | 800 ml. |

Minimal Salts

g./l.

- | | | | | |
|-----|---|---------|---|------------|
| (1) | K ₂ HPO ₄ ----- | 7.0g. | } | Solution A |
| (2) | KH ₂ PO ₄ ----- | 3.0g. | | |
| (3) | Na citrate 2H ₂ O ----- | 0.1g. | | |
| (4) | Mg SO ₄ ·7H ₂ O ----- | 0.1g. | } | Solution B |
| (5) | (NH ₄) ₂ SO ₄ ----- | 1.0g. | | |
| (6) | Agar ----- | 15.0g. | | |
| (7) | Dist. H ₂ O ----- | 900 ml. | | |

- | | | | | |
|-----|------------------------------|---------|---|------------|
| (1) | Glucose ----- | 2g. | } | Solution B |
| (2) | Dist. H ₂ O ----- | 100 ml. | | |

Add 10 ml. solu. B to solu. A before plating

Crab Infusion Agar

- | | |
|-----|---|
| (1) | 200 g. crab meat + 500 ml. H ₂ O |
| (2) | Steam 1 hr. |
| (3) | Filter |
| (4) | Add 15g. agar & make up to 1 liter |
| (5) | Add 10 g. NaCl |
| (6) | Dispense |
| (7) | Autoclave |
| (8) | Store in refrigerator until needed |

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